SCREENING FOR HEREDITARY HAEMOCHROMATOSIS

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ABSTRACT

Hereditary haemochromatosis is a common autosomal recessive disorder of iron overload in Caucasian populations. Clinical manifestations usually occur in individuals homozygous for the C282Y mutation in the *HFE* gene product and who have developed significant iron loading. Current screening methods can detect affected individuals either prior to, or early during, disease evolution, enabling early introduction of phlebotomy treatment that can normalise life expectancy. Evaluation of possible iron overload, via measurement of serum transferrin saturation and ferritin level, is the most appropriate initial test for those subjects presenting clinically for evaluation. *HFE* genotyping, when combined with serum biochemical measurements, defines the presence of likely iron overload and the underlying genetic disorder and is the preferred initial screening modality for families of an affected individual. Definitive proof of iron overload requires measurement of hepatic iron concentration or total iron burden via therapeutic phlebotomy – elevated serum ferritin level alone is not adequate. We now recognize that the natural history of HH is not as discrete as previously believed, because genetic and environmental modifiers of disease penetrance are increasingly identified as influencing the clinical expression of HH. In fact, a minority of C282Y homozygotes develop classical “iron overload disease”, although it has recently emerged that the disorder may predispose to breast and colorectal cancer. Uncertainties as to the true clinical impact of the condition at a population level lead to current recommendations of cascade screening of families of affected patients, case-finding in high-risk groups, such as patients with clinical manifestations consistent with the diagnosis, and a high level of clinical awareness in the community to facilitate early diagnosis. Generalised population screening is not presently recommended.

INTRODUCTION

Hereditary haemochromatosis (HH) is a common inherited disorder of iron metabolism affecting individuals primarily of northern European descent, which can result in iron overload, tissue injury and disease in a variable proportion of affected individuals. Whilst iron overload can result from a variety of primary or secondary causes (Table 1), the term HH is restricted to defining iron overload disease which results from mutations in a limited range of key iron metabolism genes: Type 1 HH (*HFE* gene mutations), Type 2 (hepcidin or haemojuvelin gene mutations), Type 3 (transferrin receptor 2 gene mutations), and Type 4 (ferroportin gene mutations). The mechanisms responsible for the production of iron overload in the presence of these various types of HH are reviewed elsewhere,[1] sufficed to say that all result in impaired production of the key iron regulatory hormone hepcidin, impaired iron sensing and consequential inappropriately increased iron absorption from the gastrointestinal tract and release of iron from the bone marrow iron stores.

For the purposes of this article we will hereafter only refer to *HFE*-related HH. In order to discuss screening for HH, it is first necessary to understand the natural history and clinical expressivity of HH.

DISCOVERY AND CHARACTERISATION OF *HFE*-RELATED HH

During the latter years of the 20th century, intensive genetic research led to the discovery of the candidate gene responsible for the majority of cases of for HH, termed *HFE*.[2] Two common mutations were identified in the gene product: first, tyrosine was substituted for cysteine at amino acid 282 (C282Y) and second, aspartate was substituted for histidine at amino acid 63 (H63D). Homozygosity for C282Y was observed in 85% to 90% of patients of northern European origin with typical HH,[2-5] although it was relatively rare in non-Caucasian populations.[6] The C282Y mutation in the *HFE* gene product occurs in 1 in 7 individuals of northern European descent with 1 in 200 being homozygous for the mutation.[7] The more

common H63D mutation, either alone or in homozygous form, has not been shown to be clinically important in terms of development of iron overload disease and will not be discussed further.[1]

Following the initial discovery of the *HFE* gene, several cross-sectional studies indicated a wide variation in the biochemical penetrance of the disorder, as evidenced by elevated serum ferritin levels. When stratified according to gender, biochemical iron overload was found in 70% to 88% of men and 30% to 60% of women homozygous for the C282Y mutation.[3,6-9] High clinical penetrance was found in the original cross-sectional population study in Busseleton, Western Australia. Clinical penetrance (defined as hepatomegaly, skin pigmentation, or arthropathy) was present in 50% of C282Y homozygous subjects.[5] Twenty-five percent of C282Y homozygotes had hepatic fibrosis and 10% had cirrhosis on liver biopsy. Interestingly, 25% of the homozygotes had no clinical symptoms or overt signs, with corresponding normal range serum ferritin over 4 years of follow-up.

A large Norwegian population-based study of more than 65,000 individuals aged 20 years or older reported a similar incidence of C282Y homozygosity as the Australian study, but clinical penetrance was lower.[10] Liver biopsy-proven moderate fibrosis and/or cirrhosis were seen in only 10% of cases. Fatigue and joint pain were the most common extrahepatic manifestations in 13% to 20% of newly diagnosed HH subjects, followed by impotence and diabetes mellitus (4%).[10]

A French clinic-based study also confirmed that 0.5% of the population were homozygous for C282Y, consistent with the earlier studies.[9] Ninety percent of male C282Y homozygotes had at least one clinical symptom compared with 60% of females. Family history of iron excess and chronic fatigue were common clinical features for both genders. Twenty percent of homozygous subjects had arthritis, and 5% to 10% had diabetes or increased alanine transaminase levels.

Adams et al. showed in a study of 99,711 North American subjects that the prevalence of C282Y mutation was highest in whites (0.44 %) and lowest in Asians (0.000039%). Self-reported history of liver disease was significantly higher in male C282Y homozygotes and compound heterozygotes.[6]

Contrary to the relatively high clinical expression in other population studies, Beutler et al.,[8] in a study of 41,000 patients recruited from health appraisal clinics, reported that less than 1% of C282Y homozygotes developed an overt clinical phenotype. However, the majority of clinically presenting C282Y homozygotes diagnosed prior to the commencement of this study were excluded from the analysis. Plasma type IV collagen was used as a surrogate of hepatic fibrosis and indicated that up to 25% of subjects might have liver fibrosis, although no statistically significant relationship was seen between collagen type IV and serum ferritin concentrations. Surprisingly, symptoms that represent the common clinical features of HH were no more prevalent in C282Y homozygotes than in controls.

It was clear from the cross-sectional population studies performed prior to 2005 that the clinical expressivity of HH was highly variable and most likely changed with time. Thus, resolution of the true natural history of progression of HH would require longitudinal studies.[11,12] Olynyk et al. followed the fate of 12 untreated C282Y homozygotes over 17 years. Whilst the median transferrin saturation increased from 42% to 76%, serum ferritin levels varied markedly. Fifty percent of C282Y homozygotes who underwent liver biopsy at the end of follow-up had advanced fibrosis with concurrent serum ferritin levels exceeding 500 µg/L. Clinically, only one patient had diabetes in 1981 and by the end of the study, arthritis developed in 4 of 12 patients, and hepatomegaly in 2 of 12 patients.[13] Similar findings were reported by Andersen et al. in a study of 23 untreated C282Y homozygotes over a period of 25 years, and none developed clinically overt hemochromatosis.[14]

In 2006, the US Preventive Health Services Task Force systemically reviewed HH and concluded that larger longitudinal studies would be required to consolidate understanding of the natural history of disease and the marked variance in clinical expression in terms of putative genetic and environmental modifiers.[15] These issues were comprehensively addressed by a series of Australian studies conducted on 31,192 persons of Northern European descent who were part of the prospective longitudinal study known as the Melbourne Collaborative Cohort Study.[16-18] This cohort was recruited between 1990 and 1994 through the Australian electoral roll, community announcement, and advertisement. At initial recruitment, the mean age of subjects was 55 years. Participants underwent baseline clinical assessment and biochemical evaluation. *HFE* genotyping of 31,192 baseline samples was performed. From this cohort, 1438 subjects, including all C282Y homozygotes and a stratified random sample of subjects from the remaining groups with other *HFE* genotypes, were evaluated in a follow-up study 12 years after initial recruitment. There were 203 C282Y homozygotes, 242 compound heterozygotes, 337 C282Y heterozygotes, and 147 H63D heterozygote/homozygotes identified. At baseline, 84% of male and 65% of female C282Y homozygotes had elevated serum ferritin levels, and 37% of male and 3% of female HH C282Y homozygotes had ferritin levels exceeding 1000 µg/L.[16,19] C282Y homozygous males had up to a 50% likelihood of progression to serum ferritin levels exceeding 1000 µg/L after 12 years if they had baseline serum ferritin values of 300 to 1000 µg/L. With similar baseline values, C282Y homozygous females had up to a 20% likelihood of progression to serum ferritin levels exceeding 1000 µg/L after 12 years. For both genders, the risk of biochemical progression over a 12-year period to ferritin levels exceeding 1000 µg/L was less than 15% if they had normal baseline serum ferritin values.[16,18] Furthermore, C282Y homozygotes who were likely to develop serum ferritin levels sufficient to place them at risk of iron overload–related disease generally did so by the age of 55 years.

The term “iron overload–related disease” was introduced by Allen et al.[16] in 2008 to define the presence of documented iron overload combined with clinical sequelae (cirrhosis, liver fibrosis, hepatocellular carcinoma, elevated aminotransferase levels, physician-diagnosed symptomatic hemochromatosis, or arthropathy of the second and third metacarpophalangeal joints). It is now clear that definitive diagnosis of iron overload requires more than measurement of serum ferritin levels.[1,20,28] Elevated serum ferritin levels may occur in multiple conditions, other than HH, where there is no correlation between hepatic iron stores and the ferritin level.[20,21,28] Definitive diagnosis of iron overload requires direct measurement of hepatic iron concentration or retrospective assessment of iron overload through quantitative phlebotomy therapy.[1] Direct measurement of hepatic iron concentration is achieved noninvasively through R2 magnetic resonance imaging [22] or liver biopsy.[1]

Fatigue, liver disease, and increased levels of aminotransferase are more prevalent in male C282Y homozygotes with ferritin levels exceeding 1000 μg/L compared with other *HFE* genotypes.[16] C282Y homozygous subjects with serum ferritin levels less than 1000 μg/L did not have increased symptoms or signs of iron overload disease in comparison to wild-type individuals or C282Y homozygotes who possessed normal iron studies.[23] Iron overload–related disease was present in 28% of untreated men and 1% of untreated women at the age of 65 years. Recent studies have clearly demonstrated that a classical arthropathy is associated with HH, and occurs in up to 24% of individuals, especially when the serum ferritin level exceeds 1000 μg/L.[24] A common theme to emerge from many population studies is that a threshold value for serum ferritin levels exceeding 1000 μg/L identifies those at highest risk of significant iron overload complications, especially liver fibrosis and cirrhosis.[3,25-27] Significant iron overload occurs when the hepatic iron concentration is elevated to greater than 3-times the upper limit of normal (greater than 90 μmol/g dry weight).[28] Liver fibrosis is reversible with phlebotomy therapy,[26] underscoring

the importance of diagnosis and initiating phlebotomy therapy before cirrhosis becomes established.

Apart from primary liver cancer, C282Y and H63D mutations have been implicated in development of extrahepatic cancers. H63D homozygosity was shown to be a genetic modifier of carcinogenesis in hereditary nonpolyposis colorectal cancer, where it results in a three-fold increased risk of cancer in *MMR* gene mutation carriers and earlier onset of cancer.[29] C282Y homozygosity results in a two-fold increased risk of breast cancer in women [30,31] and colorectal cancer in men and women.[30] Adding further to the emerging evidence base supporting a role for iron as a cofactor in carcinogenesis, Zacharski et al. [32] showed that a mild reduction of iron stores in normal individuals is associated with a reduced risk of cancer.

The risk of iron overload–related disease in C282Y/H63D compound heterozygotes is substantially less than that of C282Y homozygotes. Gurrin et al. [17,18] reported a longitudinal 12-year clinical follow-up study of 180 untreated compound heterozygotes (84 males) compared with 330 randomly selected wild-type control subjects. Compared with controls, mean serum ferritin levels and transferrin saturation were significantly higher for both genders, although both were within normal ranges, consistent with earlier reports.[3,33] Serum ferritin levels did not increase significantly in male or postmenopausal female compound heterozygotes after middle age. Only one male subject (who also had other liver disease risk factors) and none of the female subjects developed iron overload–related disease.

SCREENING FOR HH

The "gold standard" criteria for determination of appropriateness of generalised population screening for a disease have been defined by the World Health Organisation as: [34]

1. The condition sought should be an important health problem for the individual and community

2. The natural history of the disease should be adequately understood

3. There should be a latent or early symptomatic stage

4. There should be a suitable and acceptable screening test or examination

5. There should be an accepted treatment or useful intervention for patients with the disease

6. Facilities for diagnosis and treatment should be available

7. There should be an agreed policy on whom to treat as patients

8. Treatment started at an early stage should be of more benefit than treatment started later.

9. The cost should be economically balanced in relation to possible expenditure on medical care as a whole

10. Case finding should be a continuing process and not a once and for all project.

Hereditary haemochromatosis complies with most, but not all, of the screening criteria, as discussed below. A key issue relates to which population to screen: HH is a disorder limited to populations of northern European descent. Iron overload diseases also occur in other ethnic groups, and thus detection needs to be considered in the light of ethical decisions regarding allocation of medical resources, particularly in ethnically diverse populations such as those that exist now in many nations that were originally historically based on northern European migration.

Criterion 1. Whilst C282Y homozygosity is very common, disease sequelae which are required to meet the definition of HH are far less common. There is no doubt that some subjects with HH if left untreated can progress to serious disease which can be fatal.[1] Although the natural history of the disease is well understood, clinical penetrance is heavily influenced by genetic and environmental factors. At most, about 1% of female and 30% of male C282Y homozygous subjects will develop iron overload disease.[16-18] This equates to 1 in 20,000

females and 1 in 670 males of northern European origin suffering from iron overload disease consequential to C282Y homozygosity. The impact of the increased propensity to malignancy at a population level which is incurred through C282Y homozygosity remains to be defined.

Criterion 2. Multiple studies demonstrate that the natural history is not completely predictable. Less than 15% of adult C282Y homozygotes who possess normal serum ferritin levels will progress to ferritin levels greater than 1000 μg/L. Up to 80% of females and 50% of males who possess serum ferritin levels of at least 1000 μg/L will show reductions of ferritin levels in the absence of treatment over a 12-year time-frame. [18]

Criteria 3 and 4. Hereditary haemochromatosis has a long, asymptomatic latent period during which genetic testing for *HFE* mutations and serum iron studies (transferrin saturation and ferritin level) can be used to identify those individuals most at risk of iron overload disease. By the age of 55 years, 84% of male and 65% of female C282Y homozygotes will develop elevated serum ferritin levels, and 37% of male and 3% of female HH C282Y homozygotes will develop ferritin levels exceeding 1000 μg/L.[16,18] Symptoms and signs of iron overload disease are most likely in those whose ferritin levels exceed 1000 μg/L.

Criteria 5, 6, 7 and 8. Phlebotomy therapy for HH was first described in 1950 and has remained the cornerstone of therapy.[35] In many countries the blood transfusion services provide therapeutic phlebotomy and the blood can be utilized for various products. There are no evidence-based guidelines on therapeutic phlebotomy for HH. Treatment is conventionally commenced once serum ferritin exceeds the normal range; 200 μg/L in premenopausal women and 300 μg/L in postmenopausal women and men. The goal is to reduce the serum ferritin level to low normal range, usually 20 – 100 μg/L. In the induction phase, 1 unit (400-500 mls) of blood, equivalent to 200-250 mg of iron, can be safely removed weekly. Serum ferritin should be monitored after each 1-2 g of iron removed. Patients then undergo maintenance phlebotomy, which varies between individuals in frequency of requirement to keep serum ferritin levels in

the range 20-100 μg/L.[36-40] Levels lower than this may be associated with symptoms of iron deficiency.[40]

The benefit of phlebotomy has been clearly demonstrated in cohort studies.[42-46] Survival of clinically diagnosed HH patients who have undergone phlebotomy therapy is greater than those who are not adequately de-ironed.[42] It is also thought that pre-emptive therapy prevents complications, although treatment efficacy has never been subjected to randomized controlled studies. In the absence of complications of cirrhosis or diabetes, the life expectancy of treated patients approaches is similar to that of the age and gender-matched general population.[44,45] Phlebotomy improves liver transaminases, skin pigmentation and liver fibrosis. Up to 50% of patients with biopsy proven liver fibrosis show regression with optimal effect occurring when baseline fibrosis is mild.[26,39] Extra-hepatic manifestations such as hypogonadism, cirrhosis, deforming arthropathy and diabetes requiring insulin are usually irreversible.[46] Phlebotomy is generally accepted as being indicated in homozygotes with ferritin levels >1000 μg/L.[18] Recent studies indicate that progression of iron overload is not universal. In males, the highest risk of progression is in those subjects with ferritin levels > 1000μg/L and transferrin saturation > 95%.[18] Females have a much lower risk of progression, with the likelihood being predominantly influenced by menopausal status.

The current AASLD guidelines advocate regular phlebotomy to maintain the serum ferritin between 50 and 100 μg/L in asymptomatic individuals with homozygous HH, markers of iron overload, and histological evidence of potentially toxic levels of hepatic iron.[40] However, more recent Australian data suggests an alternative approach might be to monitor HH subjects with serum ferritin levels between the upper limit of normal and 1000 μg/L.[16,18] These studies demonstrated that the risk of development of iron-overload disease in C282Y homozygous subjects with ferritin levels in the range between upper limit of normal and 1000 μg/L was no greater than that observed in C282Y homozygotes with normal ferritin or wild-

type controls.[17,18] Less that 50% of male C282Y homozygotes and less than 20% of female C282Y homozygotes with serum ferritin levels less than 1000μg/L at the age of 55 years progress to levels greater than 1000μg/L by the age of 67 years.[18]

Criterion 9. The ultimate determinant of implementation of a publicly funded screening program is cost. There is no doubt that genetic and/or biochemical screening will discover C282Y homozygotes at various stages of disease evolution, some of whom will benefit from early intervention. Early cost-effectiveness modeling attempts were based on higher levels of disease expression than is apparent from the more recent population studies.[16,47,48]

Cascade screening of male individuals within affected families following the initial diagnosis of a proband has been demonstrated to be the most cost-effective strategy (41,000 €/life year gained (LYG)), whereas sequential population screening using either biochemical or genetic testing cost in the order of 124,000 or 161,000 €/LYG, respectively.[49] Screening within families provides a 25% likelihood of returning other affected individuals.[49-51] Gagne et al (2007) concluded that provided there was at least 70% biochemical expression with subsequent development of clinical sequelae in 5% of these individuals, screening was cost-effective.[48] These assumptions most closely reflect the natural history of disease progression in males.[16] Due to the limited expressivity in females compared with males, Phatak et al (2008) also recommended screening be focused on males over the age of 25 years and of northern European descent, or those with a family history of HH.[47]

A high level of clinical awareness which leads to "case-finding" for HH is advocated in individuals presenting with clinical manifestation known to be associated with HH. These include chronic liver disease, diabetes mellitus and arthritis.[1,16] Routine screening *per se* has not been proven to be beneficial in subjects with diabetes mellitus.[52] Up to 24% of subjects with HH have a typical bilateral peripheral arthropathy affecting metacarpophalangeal joints, hips or knees.[23]

In summary, true “screening” is currently advocated only in the setting of cascade screening of families. Thereafter, males of northern European origin should be considered a risk group that is worthy of close scrutiny for assessment of iron status during early to mid adult life, often in the setting of routine clinical assessments. A high level of awareness of the clinical manifestations of HH, together with the high variability of clinical expressivity, is required to guide clinicians in the appropriate detection of affected HH individuals in the most timely fashion.

Table 1. Classification of iron overload states

Hereditary Haemochromatosis

- **Type 1 (*HFE*-related)**
  - C282Y homozygous (common, high risk for iron overload)
  - C282Y/H63D compound heterozygous (common, low risk for iron overload)
  - Other *HFE* mutations (common, low risk for iron overload)
- **Type 2 (juvenile hemochromatosis)**
  - **A.** hemojuvelin (*HJV*) mutations (rare, high risk for iron overload)
  - **B.** hepcidin (*HAMP*) mutations (rare, high risk for iron overload)
- **Type 3 (*TFR2* mutations)** (rare, high risk for iron overload)
- **Type 4 (ferroportin mutations)** (rare, high risk for iron overload)
  - **A.** loss-of-function
  - **B.** gain-of-function

Secondary Iron Overload

- Iron-loading anemias
- Parenteral iron overload
- Long-term hemodialysis
- Chronic liver disease
  - Alcoholic liver disease
  - Hepatitis B or C
  - Porphyria cutanea tarda
- Nonalcoholic steatohepatitis

Miscellaneous

- Congenital alloimmune hepatitis (neonatal iron overload)

African iron overload

Aceruloplasminemia

Atransferrinemia

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